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# ULTRASTRUCTURE OF SPOROZOITES OF *SCHELLACKIA GOLVANI* (EIMERIORINA: LANKESTERELLIDAE) IN THE GREEN ANOLE, *ANOLIS CAROLINENSIS*

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**Abstract**—KLEIN T. A., AKIN D. C. and YOUNG D. G. 1992. Ultrastructure of sporozoites of *Schellackia golvani* (Eimeriorina: Lankesterellidae) in the green anole, *Anolis carolinensis*. *International Journal for Parasitology* **22**: 767–772. In the lizard host, *Schellackia golvani* sporozoites were observed in parasitophorous vacuoles of the polymorphonuclear series of leukocytes. Surrounding the parasite in the parasitophorous vacuole are numerous vesicles, intravascular tubules and electron-dense granules. The parasite envelope consists of a double membrane. A cytostome (micropyle), a conoid and apical rings are present. Paralamellate bodies, mitochondria, nucleus, nucleolus, rhopteries, micronemes and a single non-membrane-bound electron-lucid body were identified. The sporozoite subcellular morphology of *S. golvani* is similar to other sporozoan species belonging to the genera of *Schellackia*, *Haemogregarina*, *Lankesterella*, *Eimeria* and *Toxoplasma*.

INDEX KEY WORDS: Ultrastructure; sporozoites; *Schellackia golvani*; *Anolis carolinensis*; leukocytes.

## INTRODUCTION

*Schellackia golvani* Rogier & Landau, 1975 and *Schellackia occidentalis* were observed in bloodfilms of wild-collected lizards, *Anolis carolinensis* and *Sceloporus undulatus*, respectively, in Florida. Experimental transmission studies resolved the species status of the two *Schellackia* parasites found in these Florida lizards (Klein, Young, Greiner, Telford & Butler, 1988). The studies showed that parasite transmission is accomplished by ingestion of infected hematophagous arthropods, of which a wide variety are capable of harboring *Schellackia* sporozoites, the infective stage.

Electron microscopy studies of *Schellackia* parasites and biological studies of their relationship with the saurian hosts may lead to a better understanding of the evolution of hemococcidians and may show how they became adapted to an alternating life cycle in a vertebrate host. Ultrastructural studies determining morphological relationships have been done on a number of coccidians and sporidians, but there are only two studies on the sporozoite stage of *Schellackia*, *S. occidentalis* by Sinden & Moore (1974) and *S. agamae* by Paperna & Ostrovska (1989). Data from this present study are compared with those of other hemosporina to identify differences and similarities among species and related groups.

## MATERIALS AND METHODS

Sporozoites of *S. golvani* were obtained by clipping the tail of laboratory-infected *A. carolinensis* and placing drops of blood directly into a fixative of 2% glutaraldehyde buffered with 0.2 M-sodium cacodylate (pH 7.2). The blood was fixed for 30 min at room temperature, rinsed, and placed in buffered 1% osmium tetroxide solution for 30 min. After rinsing with deionized water, the blood was pelletized and embedded in 2% agar, dehydrated in a graded ethanol-acetone series and embedded in Spurr's resin (Spurr, 1969). Thin sections were cut using an LKB Ultratome III, picked up on slot copper grids, stained with uranyl acetate and lead citrate and viewed using a JEOL 100CX electron microscope. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide For the Care and Use of Laboratory Animals*, NIH Publication 85-23.

## RESULTS

The ultrastructure of intracellular *S. golvani* sporozoites is similar to that described for *S. occidentalis*

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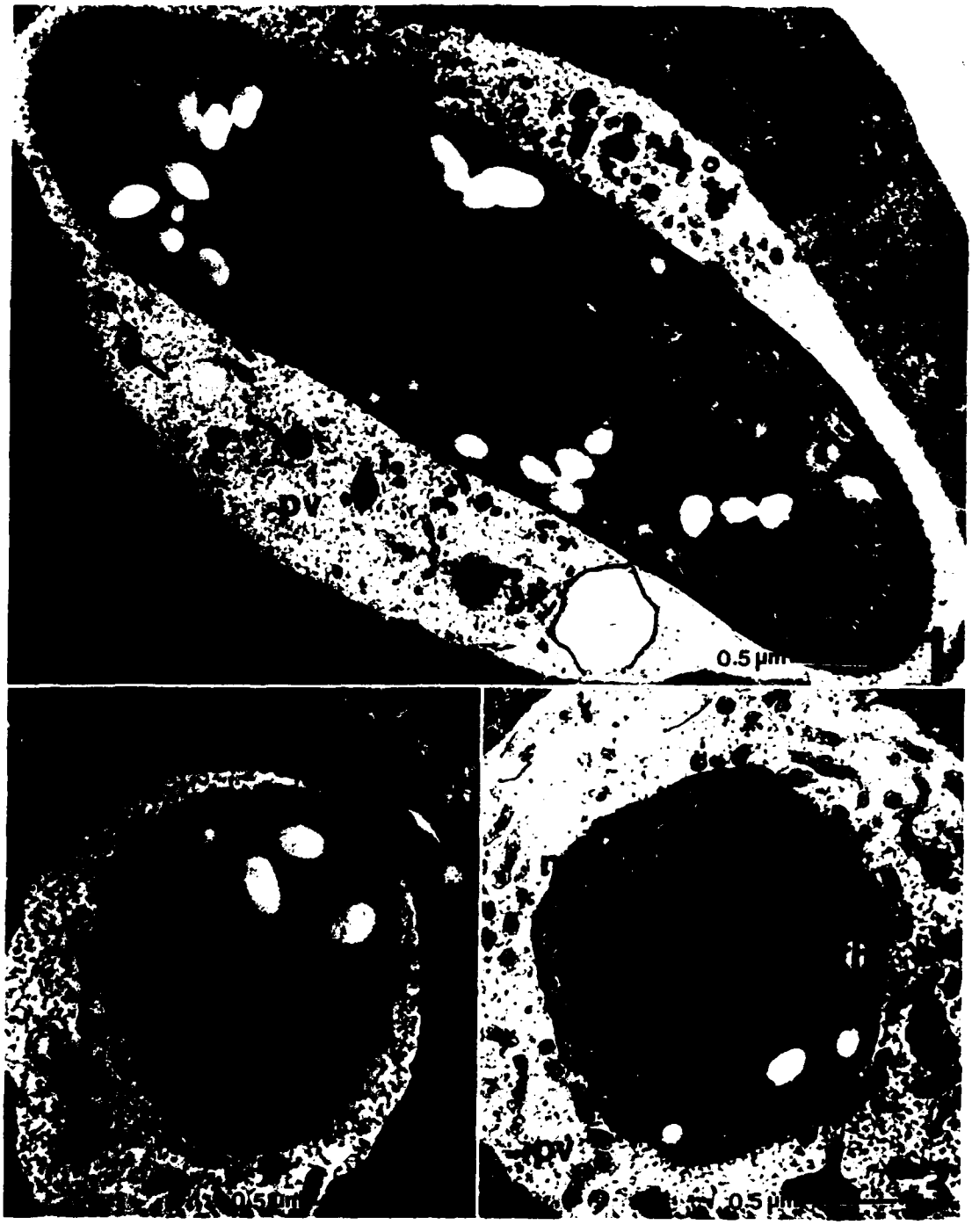



FIGS. 1–3.

and *S. agamae* by Sinden & Moore (1974) and by Paperna & Ostrovska (1989). The intracellular sporozoites were found in large parasitophorous vacuoles (Figs. 1–3, 4a). Intravascular tubules (sinuous villus-like projections) extend inwardly from the margin of the parasitophorous vacuoles and appear to be in contact with the sporozoite (Fig. 3). Electron-dense granules were observed in the parasitophorous vacuoles (Figs. 1, 4a).

The anterior and posterior ends of *S. golvani* sporozoites are bluntly truncated (Figs. 1, 4a). Posteriorly, the sporozoites are not recurved as described for *S. occidentalis*. In both light microscopy and TEM studies, the PMN cells were not distorted by the sporozoite. The sporozoites are often associated with the host cell nucleus and appear to be surrounded by it on three sides. The sporozoite pellicle consists of two unit membranes; the inner membrane being less electron dense than the outer membrane (Fig. 7). Along the lateral edge, the outer membrane invaginates through breaks in the inner membrane to form the cytostome (Fig. 4). A conoid and two polar rings were observed at the anterior end of the sporozoite (Fig. 5).

There were up to five rhopteries and numerous micronemes scattered throughout the cytoplasm and extending from the posterior of the sporozoite (Figs. 1–3, 4a). A prominent spherical non-membrane-bound electron-lucid body, located centrally to subcentrally, appeared to be associated with the nucleus (Figs. 1, 3, 4a). The centrally located nucleus is large, spherical and contains a small amount of electron-dense heterochromatin along the periphery (Figs. 1, 4a). A prominent nucleolus is present (Fig. 4a). Several mitochondria were observed in the anterior and posterior of the sporozoite adjacent to the nucleus (Fig. 1, 4a). Membrane-bound paralamellate bodies were present (Figs. 2, 6).

## DISCUSSION

Sporozoites of *S. golvani* were observed in the PMN series of leukocytes in *A. carolinensis* from Florida. Although Rogier & Landau (1975) found *S. golvani* sporozoites in all forms of leukocytes in *Anolis marmoratus*, PMN cells were most frequently infected. Other species of *Schellackia* invade leukocytes, erythrocytes or both (Lainson, Shaw & Ward, 1976). Paperna & Ostrovska (1989) reported that *S. agamae* invade hepatic cells as well as macrophages and erythrocytes. They hypothesized that this is a defined developmental stage for sporozoites of *S. agamae*, not a diapausing stage. Sporozoite invasion of hepatocytes has not been previously reported for other *Schellackia* spp., but this stage of development may have been overlooked.

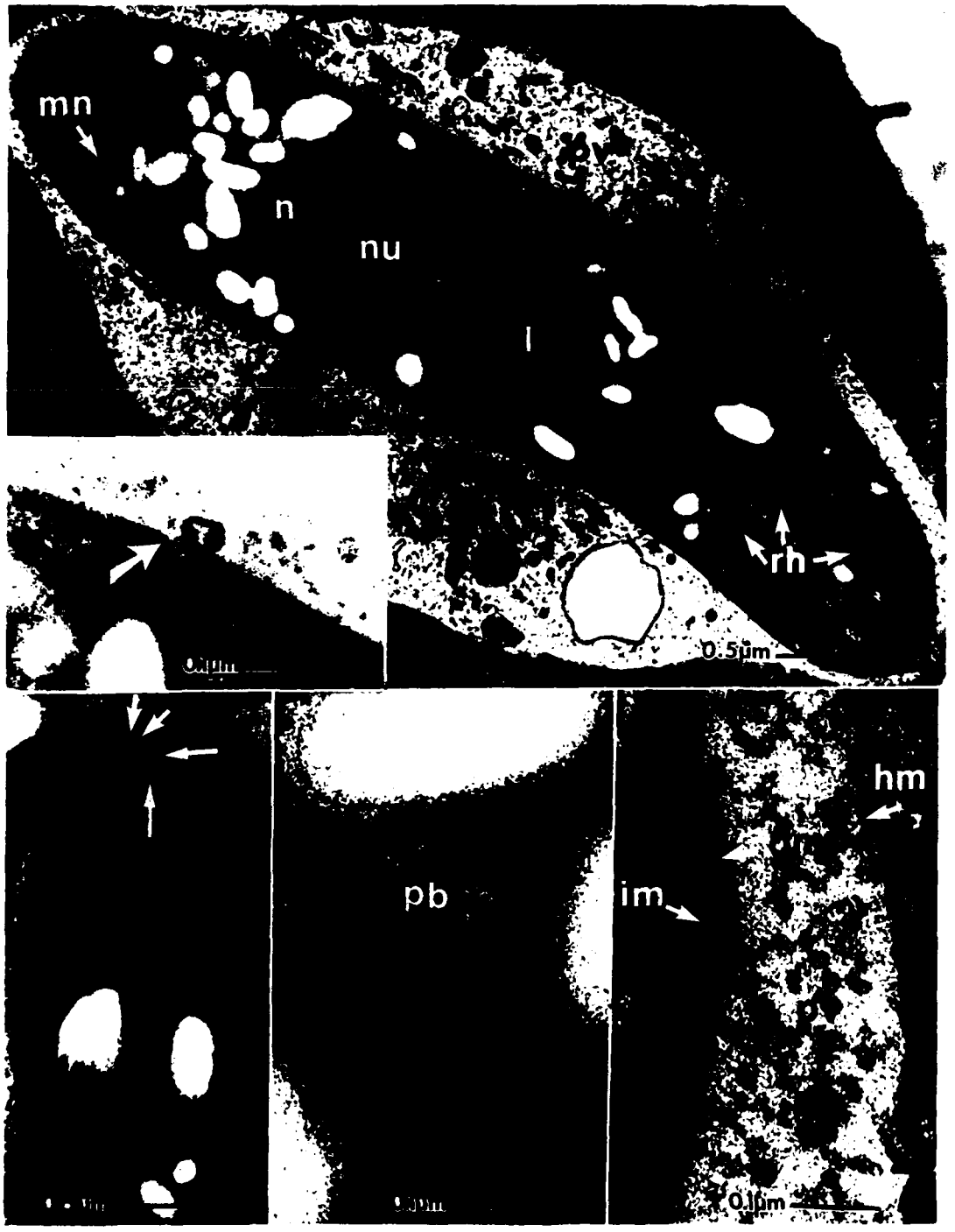
*Schellackia golvani* resembled blood stages of other *Schellackia* spp., but unlike *S. occidentalis*, *S. agamae*, *Haemogregarina hamata* and *Lankesterella minima*, did not exhibit a recurved tail-like extension (Rogier, 1977; Sinden & Moore, 1974; Garnham, 1950; Tse, Barta & Desser, 1986).

Sporozoites of *S. golvani* are contained in parasitophorous vacuoles as are sporozoites of *Eimeria* spp., *Lankesterella* spp., *S. occidentalis* and *S. agamae* (see Roberts & Hammond, 1970; Roberts, Hammond & Speer, 1970; Stehbens, 1966; Sinden & Moore, 1974; Paperna & Ostrovska, 1989). The vacuole membrane of *S. golvani* appears to be broken along its margin and has intravascular tube-like membranes extending from its margin (stereocilia of Sheffield & Melton, 1968). Intravascular tubules extending from the host vacuole membrane surrounding *S. golvani* sporozoites did not appear to be confluent with the parasite membrane and are similar to the intravascular tubules (vesicles) observed in *Lankesterella hylae* by Stehbens (1966). Intravascular tubules originating from the vacuole membrane were also observed in *Toxoplasma gondii*. Sibley, Krahenbuhl, Adams & Weidner (1986) and Sibley & Krahenbuhl (1988) indicated that these intravascular tubules are secreted by the parasite within 5 min of invading the cell. These tubule-shaped membranes originate at the

FIG. 1. Longitudinal section of a *Schellackia golvani* sporozoite in a polymorphonuclear leukocyte (h) of *Anolis carolinensis* showing the parasitophorous vacuole (pv) with electron-dense granules (black arrows), nucleus (n), non-membrane bound electron-lucid body (l), mitochondria (m) and rhopteries (rh).

FIG. 2. Cross sections of *Schellackia golvani* in a parasitophorous vacuole (pv) of a polymorphonuclear leukocyte (h). Rhopteries (rh), numerous micronemes (mn) and paralamellate bodies (pb) are scattered throughout the cytoplasm.

FIG. 3. Cross section of *Schellackia golvani* in a polymorphonuclear leukocyte showing rhopteries (rh), mitochondria (m) and numerous micronemes (mn). A non-membrane-bound refractile body (l), corresponding to the refractile body of living sporozoites, is subcentrally located in the cytoplasm. The parasitophorous vacuole (pv) contains numerous electron-dense inclusions and intravascular tubules (s) (stereocilia), apparently originating from the host tissue.




FIGS. 4–7.

membrane of the parasite and extend to the host parasitophorous membrane and may function in evasion of microbicidal events. The morphology of parasitophorous vacuoles in red blood cells infected with *L. hylae* sporozoites was different from those in phagocytes, indicating that the function of these membranes may be an evasion function as hypothesized by Sibley & Krahenbuhl (1988).

The cytostome and double membrane pellicle of *S. golvani* are also found in *Eimeria*, *Lankesterella* and *Toxoplasma*. Sinden & Moore (1974) and Paperna & Ostrovska (1989) did not observe a cytostome in *S. occidentalis* or *S. agamae* but Sinden & Moore (1974) believed it was present based on the presence of this structure in other Sporozoa.

The internal anterior morphology of *S. golvani* is similar to that observed in *S. occidentalis*, *S. agamae*, and members of the genera *Eimeria* and *Lankesterella*. A centrally located non-membrane-bound electron-lucid body associated with the nucleus corresponds to the refractile body observed in living sporozoites. This body is observed in *S. occidentalis*, *S. agamae*, *Lankesterella hylae* (2) and *Eimeria* spp. This structure is believed to be lipid or carbohydrate and may serve as an energy reserve.

Paralamellate bodies were seen in both *S. golvani* and *S. occidentalis* by Sinden & Moore (1974). Similar non-membrane-bound "closely arranged rows of parallel membranes" were observed in *L. hylae* by Stehbens (1966). The functions of these organelles are not known; they were not observed in *S. agamae* or members of the genera *Eimeria* or *Toxoplasma*.

In conclusion, the sporozoites of *S. golvani* closely resemble those of *S. occidentalis* and *S. agamae*. The principal differences are the lack of a recurved tail in *S. golvani* and a cytostome in *S. occidentalis*. There are also many similarities to sporozoites of members of the genera *Eimeria*, *Toxoplasma*, and *Lankesterella*, but *S. golvani* resembles the latter more closely.

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FIG. 4. (a) Longitudinal section of *Schellackia golvani* sporozoite in a parasitophorous vacuole (pv) of a polymorphonuclear leukocyte (h) of *Anolis carolinensis*. A micropore (arrow), nucleus (n) with an electron-dense nucleolus (nu), non-membrane-bound electron-lucid body (l) corresponding to the refractile body of living sporozoites, electron-dense inclusions (small arrow), rhopteries (rh), mitochondria (m) and micronemes (nm) are shown. (b) Enlargement of the micropore (arrow) found during serial sectioning.

FIG. 5. Slightly oblique longitudinal section of the conoid (large arrows) and polar rings (small arrows) of *Schellackia golvani* sporozoite.

FIG. 6. Paralamellate bodies (pb) in *Schellackia golvani* sporozoite.

FIG. 7. Electron micrograph illustrating the outer membrane (om) and less distinct inner membrane (im) of a *Schellackia golvani* sporozoite, parasitophorous vacuole (pv) and host membrane (hm).

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